A role for heterodimerization of μ and δ opiate receptors in enhancing morphine analgesia

Ivone Gomes*†, Achla Gupta*†, Julija Filipovska*, Hazel H. Szeto‡, John E. Pintar§, and Lakshmi A. Devi*†¶

†Department of Pharmacology and Biological Chemistry, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029; *Department of Pharmacology, New York University School of Medicine, New York, NY 10016; ‡Department of Pharmacology, Weill Medical College of Cornell University, New York, NY 10021; and §Department of Neuroscience and Cell Biology, University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School, Piscataway, NJ 08854

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Opiates such as morphine are the choice analgesic in the treatment of chronic pain. However their long-term use is limited because of the development of tolerance and dependence. Due to its importance in therapy, different strategies have been considered for making opiates such as morphine more effective, while curbing its liability to be abused. One such strategy has been to use a combination of drugs to improve the effectiveness of morphine. In particular, δ opioid receptor ligands have been useful in enhancing morphine's potency. The underlying molecular basis for these observations is not understood. We propose the modulation of receptor function by physical association between μ and δ opioid receptors as a potential mechanism. In support of this hypothesis, we show that μ-δ interacting complexes exist in live cells and native membranes and that the occupancy of δ receptors (by antagonists) is sufficient to enhance μ opioid receptor binding and signaling activity. Furthermore, δ receptor antagonists enhance morphine-mediated intrathecal analgesia. Thus, heterodimeric associations between μ-δ opioid receptors can be used as a model for the development of novel combination therapies for the treatment of chronic pain and other pathologies.

Opioid receptors belong to the rhodopsin family of G protein-coupled receptors (GPCRs). Like many GPCRs, these receptors were thought to function as single units. This notion has been revised in recent years by a number of studies showing that GPCRs associate with each other to form dimers and/or oligomers (1–3). Of particular significance are the studies with rhodopsin, a prototypical member of the GPCR family, where infrared-laser atomic-force microscopy of native mouse disk membranes showed the receptors to be arranged in crystalline arrays of dimeric units (4, 5). Also, data from x-ray crystallographic studies with rhodopsin (6, 7) and the N terminus of metabotropic glutamate receptors (8), support the notion that dimerization is an integral feature of these receptors and could play a key role in modulating their function.

The three types of opioid receptors (μ, δ, and κ) have been shown to associate with each other in a homotypic or heterotypic fashion when expressed in heterologous cells (9–11). Furthermore, heterotypic interactions appear to alter the ligand-binding and signaling properties of these receptors (12). However, until now, it was not clear whether these interactions occurred in live cells and in endogenous tissues and whether they were physiologically relevant. In this study, we addressed these questions by using multiple approaches. We used the bioluminescence resonance energy transfer (BRET) assay to show that μ and δ receptors interact in living cells. In addition, we show that signaling by clinically relevant drugs, such as morphine, fentanyl, and methadone can be enhanced by δ receptor ligands. This potentiation of μ receptor signaling by the δ receptor antagonist is seen in membranes from WT mice and not in membranes from δ receptor lacking mice (δ k/o). Finally, we show that morphine-mediated intrathecal analgesia is potentiated by a δ receptor antagonist. Taken together, our results suggest that μ–δ receptor interactions lead to profound modulation of μ receptor signaling by δ antagonists.

Methods

BRET Assay. HEK-293 cells were transfected with μ luciferase (Luc) and δ yellow fluorescent protein (YFP), or were cotransfected with μLuc and δYFP or μLuc and CCR5YFP by using Lipofectamine as per manufacturer's protocol. In a parallel set of experiments, cells were transfected with δLuc and μYFP, or were cotransfected with δLuc and μYFP or δLuc and CCR5YFP. After 48 h, cells were washed with PBS, were suspended to $\approx 1–2 \times 10^6$ cells per ml, and were treated with coelenterazine (5 μM final concentration). Light emission was monitored with a close excitation slit every 0.5 sec from 420 to 590 nm at 5-nm intervals by using a FluoroMax-2 spectrometer.

Immunoprecipitation with mAbs. mAbs were raised against N-terminal 14–30 amino acids of mouse μ or N-terminal 3–17 amino acids of mouse δ opioid receptors by using standard procedures. These antibodies were found to be highly selective for their respective receptors exhibiting negligible crossreactivity to other opioid receptor subtypes (A.G., I.G., F. Decaillot, and L.A.D., unpublished work). Membranes prepared from spinal cords of WT/δ knockout mice were solubilized with 5% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate in 50 mM Tris·HCl, pH 7.5, containing a protease inhibitor mixture (Sigma, catalog no. P-8340) and were subjected to immunoprecipitation with 1 μg of μ or δ mAb. Immunocomplexes were bound to anti-mouse IgG coupled to agarose beads and were analyzed by Western blot analysis using δ polyclonal antibodies (Chemicon) or μ polyclonal antibodies (a gift from T. Cote, Uniformed Services University of the Health Sciences, Bethesda) as described (13).

Ligand-Binding Assays. Chinese hamster ovary (CHO) cells stably expressing μ receptors, coexpressing μ and δ receptors or SK-N-SH cells endogenously expressing μ and δ receptors were plated into poly-L-lysine-coated 24-well plates. Cells were incubated with increasing doses of ^{3}H-[D-Ala^2,*N*-MePhe^4,Gly^5-ol]enkephalin (DAMGO) or ^{3}H-morphine in the absence or presence of 10 nM Tyr-Ticψ-(CH2NH)-Phe-Phe (TIPPψ), ICI 174,864, naltriben, or deltorphin II (Delt II) for 2 h at 37°C. Cells were washed in ice-cold 50 mM Tris-HCl, pH 7.5, were solubilized, and radioactivity was detected in a liquid scintillation counter.

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Abbreviations: GPCR, G protein-coupled receptor; BRET, bioluminescence resonance energy transfer; CHO, Chinese hamster ovary; DAMGO, [D-Ala^2,*N*-MePhe^4,Gly^5-ol]enkephalin; YFP, yellow fluorescent protein; Delt, deltorphin; Luc, luciferase; TIPPΨ, Tyr-TicΨ-(CH2NH)-Phe-Phe.

¶To whom correspondence should be addressed. E-mail: lakshmi.devi@mssm.edu.

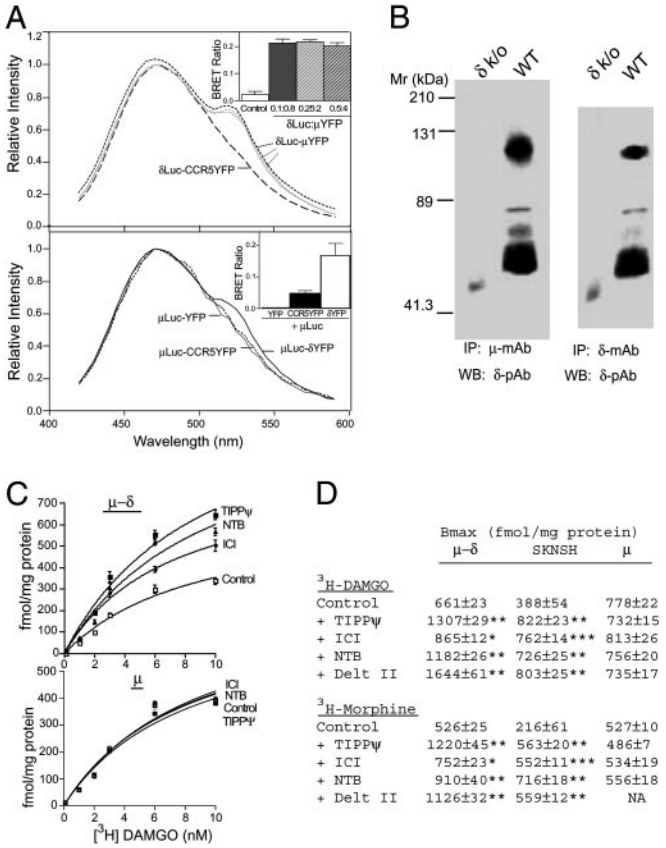

Fig. 1. μ and δ receptor interactions in heterologous cells and endogenous tissue. (*A*) BRET assay shows significant energy transfer between μ and δ receptors in live cells. (*Upper*) Light emission was monitored for cells transfected with δLuc and CCR5YFP (long black dash) or various amounts of δLuc:μYFP; 0.1:0.8 (gray line), 0.25:2 (gray dash), and 0.5:4 (small black dash). Spectra in *A* expressed as BRET ratio, defined as the ratio of the area under the curve of light emission for δLuc:μYFP and the curve of light emission for δLuc alone, is shown in the *Inset*. (*Lower*) Light emission was monitored for cells coexpressing μLuc with either CCR5YFP (dash), or δYFP (black), or YFP (gray). Spectra in *B* expressed as BRET ratio is shown in *Inset*. (*B*) μ and δ receptor complexes can be isolated from spinal cord membranes. Solubilized spinal cord membranes from WT mice or mice lacking δ receptors (δ k/o) were subjected to immunoprecipitation by using 1 μg of anti-μ or anti-δ mAbs (μ-mAb or δ-mAb). Western blotting of immunocomplexes isolated by using anti-μ or anti-δ mAbs with δ polyclonal antibodies (Chemicon) detected δ receptors in membranes from WT mice but not from mice lacking δ receptors. Western blotting of immunocomplexes isolated by using anti-δ mAbs antibodies with μ polyclonal antibodies (gift from T. Cote) detected μ receptors in membranes from WT mice but not from mice lacking δ receptors (data not shown). (*C* and *D*) δ antagonists modulate the binding of ^{3}H-DAMGO and ^{3}H-morphine. (*C*) CHO cells stably expressing μ receptors or coexpressing μ and δ opioid receptors were incubated with increasing doses of ^{3}H-DAMGO in the absence or presence of various ligands (10 nM). The incubations were carried out for 2 h at 37°C. Cells were washed in ice-cold 50 mM Tris·HCl, and radioactivity bound was detected as described (13). Results are mean ± SEM of three experiments in triplicate. (*D*) CHO cells stably expressing μ receptors, coexpressing μ and δ opioid receptors or SK-N-SH cells endogenously expressing these receptors were incubated with increasing doses of ^{3}H-DAMGO or ^{3}H-morphine in the absence or presence of various ligands (10 nM), and the radioactivity bound was detected as described (13). Results are mean ± SEM of three experiments in triplicate. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, Dunnett's test. NA, not available.

[^{35}S]GTPγS Binding Assay. CHO cells stably coexpressing μ and δ receptors or SK-N-SH cells endogenously expressing μ and δ receptors were permeabilized in 0.5% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate. Ligand-mediated increase in [^{35}S]GTPγS binding in response to increasing doses of

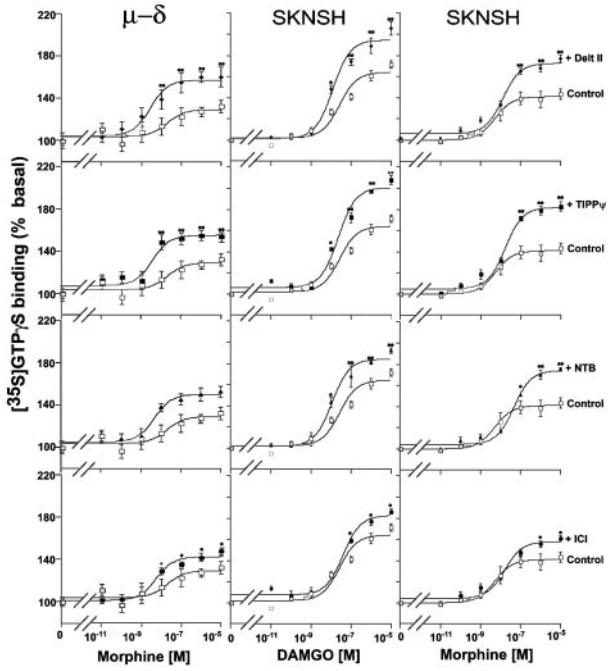

Fig. 2. δ receptor ligands enhance μ receptor activity in cell lines expressing μ-δ receptors. Agonist-mediated [^{35}S]GTPγS binding in CHO cells coexpressing μ and δ receptors or in SK-N-SH cells endogenously expressing μ and δ receptors. Cells were permeabilized with 0.5% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate in 50 mM Tris·HCl, pH 7.5, and were treated with indicated doses of morphine or DAMGO with or without 10 nM Delt II, TIPPψ, naltriben, or ICI 174,864, and the [^{35}S]GTPγS binding was measured as described (13). Basal values, determined in the absence of the μ agonist, but in the presence of δ ligands, were taken as 100%. Results are the mean ± SEM of three experiments in quadruplicate. **, $P < 0.005$; *, $P < 0.01$, Student's *t* test.

μ agonist in the presence or absence of a fixed dose of δ ligands in permeabilized cells or spinal cord membranes from WT or δ knockout mice were carried out essentially as described (13). The lowest dose of the δ ligand that gave a near maximal effect was chosen after carrying out dose–response studies. The δ agonist, Delt II, at the concentration used in this study, did not cause a significant change in basal values. Basal values for μ-δ cells were 9.2 ± 0.19 and 9.14 ± 0.15 fmol per 10^7 cells in control and Delt II respectively, and for SK-N-SH cells were 10.07 ± 0.31 and 9.97 ± 0.12 fmol per 10^7 cells in control and Delt II, respectively.

Intracellular cAMP Assay. SK-N-SH cells endogenously expressing μ and δ receptors were treated with increasing doses of morphine in the absence or presence of 10 nM TIPPψ for 20 min at 37°C. The intracellular cAMP levels after agonist treatment were measured by an RIA as described (9).

Analgesia Assay. Mice were injected intrathecally with 0.3 nmol of morphine in the absence or presence of 2 nmol of TIPPψ (4 μl per mouse). Antinociception was measured by the radiant tail-flick assay as described (14). Light intensity was adjusted such that baseline latencies ranged between 2.5 and 3.5 sec. Analgesia was defined as a latency response of greater than two times the baseline latency for an individual animal. To avoid tissue damage, a cutoff of 10 sec was used. Data obtained was expressed in terms of percent maximal possible effect.

Results

Examination of μ–δ Interactions in Live Cells. To examine μ–δ receptor interactions in live cells, we used the BRET assay (13).

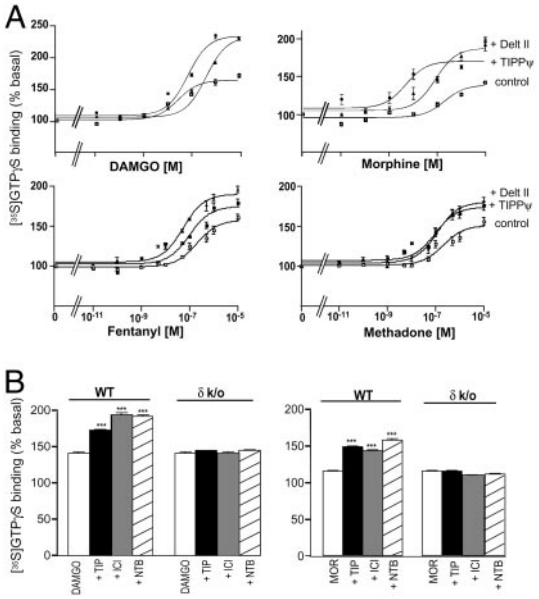

Fig. 3. A variety of δ ligands enhance μ receptor signaling mediated by clinically relevant drugs. (*A*) Membranes from mouse spinal cords were treated with the indicated doses of DAMGO, morphine, fentanyl, or methadone with or without 10 nM of either TIPPψ or Delt II, and the [^{35}S]GTPγS binding was measured as described (13). Basal values, determined in the absence of μ agonist, but in the presence of δ ligands, were taken as 100%. Results are the mean ± SEM of four experiments in quadruplicate. (*B*) Membranes from WT mice and mice lacking δ receptors (δ k/o) were treated with 10^{-7} M DAMGO (*Left*) or morphine (*Right*) with or without 10 nM TIPPψ (TIP), ICI174,864 (ICI), or naltriben, and [^{35}S]GTPγS binding was measured as described (13). Basal values, determined in the absence of μ agonist, but in the presence of δ ligands, were taken as 100%. Results are the mean ± SEM of three experiments in quadruplicate. ***, $P < 0.001$, Student's *t* test.

For this assay, luciferase-tagged δ or μ receptors were coexpressed with YFP-tagged μ or δ receptors, respectively, and the relative energy transfer between the two receptors was measured. We found a significant increase in BRET signal when either combination of μ-δ opioid receptors were coexpressed (Fig. 1*A*). A BRET signal was not seen when luciferase-tagged μ or δ receptors were coexpressed with YFP-tagged CCR5 chemokine receptors, suggesting the specificity of this interaction (Fig. 1*A*). The interaction is not due to receptor overexpression because lowering the level of receptor expression (to near endogenous levels, ≈200–300 fmol/mg protein obtained in cells cotransfected with 0.1 μg of δ luciferase and 0.8 μg of μYFP) had no significant effect on the intensity of the BRET signal. The signal remained constant as long as the ratio of the donor- and acceptor-tagged opioid receptors was kept constant (Fig. 1*A*). We also found that the BRET ratio was not affected by treatment with ligands to one receptor or to both receptors (data not shown). These results indicate that μ and δ receptors are within 100 Å in live cells, which is a proximity close enough to allow direct receptor-receptor interactions.

Isolation of μ-δ Immunocomplexes from Spinal Cord Membranes. Biochemical techniques such as differential epitope-tagging and immunoprecipitation have been used to study GPCR associations, primarily by coexpression in heterologous cells (11, 12). To examine μ–δ receptor associations in endogenous tissue, we raised selective mAbs directed toward the N-terminal region of mouse μ or δ opioid receptors (A.G., I.G., F. Decaillot, and L.A.D., unpublished work). These antibodies are receptor-type-selective (because the μ receptor antibody does not recognize δ or κ receptors and the δ receptor antibody does not recognize μ or κ receptors), and can recognize native receptors in endogenous tissue (A.G. and L.A.D., unpublished work). We used these antibodies to isolate μ–δ receptor complexes from spinal cord membranes (that have been shown to express both receptors within the same neuron; ref. 15). As shown in Fig. 1*B*, we were able to isolate μ–δ receptor complexes from membranes of WT mice but not of mice lacking δ receptors. These results indicate that μ–δ receptor complexes are present in endogenous tissue such as spinal cord, which are known to be involved in pain transmission.

Ligand-Binding Properties of μ–δ Heterodimers. We examined the significance of μ–δ interactions in modulating μ receptor function by using heterologous cells or neuroblastoma cells coexpressing these receptors with regards to the ability of δ ligands to modulate the binding of μ receptor ligands (13). We find a

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Table 1. δ antagonists modulate the μ agonist-mediated increased in [^{35}S]GTPγS binding

	EC_{50}, nM			E_{max}, percent basal		
	μ–δ	SK-N-SH	Spinal cord	μ–δ	SK-N-SH	Spinal cord
Morphine	20.3 ± 0.44	5.3 ± 1.3	198 ± 2.1	133 ± 5.9	144 ± 5.1	149 ± 4.6
Plus TIPPψ	3.5 ± 0.28**	16.7 ± 1.4**	4.7 ± 3.4**	154 ± 0.3**	183 ± 4.6**	197 ± 6.1**
Plus ICI	5.6 ± 0.17**	16.2 ± 1.5**	16.4 ± 2.0**	148 ± 2.7*	161 ± 2.4**	177 ± 5.4**
Plus Naltriben	4.7 ± 0.17**	46.7 ± 1.3**	37 ± 1.6**	153 ± 5.5**	175 ± 1.7**	184 ± 2.0**
Plus Delt II	—	—	83 ± 1.6**	—	—	193 ± 4.4**
DAMGO	—	27 ± 1.8	27 ± 1.6	—	172 ± 3.7	171 ± 3.6
Plus TIPPψ	—	23 ± 1.5	70 ± 1.5**	—	208 ± 4**	230 ± 2.7**
Plus ICI	—	40 ± 1.4**	3.7 ± 1.8**	—	187 ± 2.5**	195 ± 5.0**
Plus Naltriben	—	11 ± 1.3**	14 ± 1.5**	—	192 ± 2.5**	212 ± 7.6**
Plus Delt II	—	—	414 ± 1.4**	—	—	230 ± 2.4**
Fentanyl	—	—	197 ± 11	—	—	159 ± 6.9
Plus TIPPψ	—	—	9.6 ± 2.1**	—	—	173 ± 3.5**
Plus Delt II	—	—	53.3 ± 1.2**	—	—	181 ± 7.3**
Methadone	—	—	206 ± 18	—	—	149 ± 2.2**
Plus TIPPψ	—	—	93 ± 1.7**	—	—	162 ± 3.3**
Plus Delt II	—	—	115 ± 12**	—	—	173 ± 2.1**

—, Not available. Membranes from mouse spinal cords were treated with DAMGO, morphine, fentanyl, or methadone with or without 10 nM of δ ligands, and the extent of [^{35}S]GTPγS binding was measured as described (13). Data were analyzed by using PRISM 2.0 (GraphPad, San Diego) to determine the EC_{50} and E_{max}. Results are the mean ± SEM of four experiments performed in quadruplicate. *, $P < 0.05$; **, $P < 0.01$, Dunnett's test.

substantial increase in the binding of μ ligands by a variety of δ ligands, including agonist (Delt II), peptide antagonist (TIPPψ), nonpeptide antagonist (naltriben), and inverse agonist (ICI 174,864) (Fig. 1 *C* and *D*). The increase is seen with morphine (a clinically relevant drug, Fig. 1*D*) as well as DAMGO (a highly selective μ receptor ligand); the increase is not seen in cells expressing only μ receptors (Fig. 1 *C* and *D*). Taken together, these results suggest that the coexpression of μ and δ receptors is required for the observed enhancement of μ agonist binding by δ receptor ligands, and that the occupancy of the δ receptor is sufficient to see this effect.

Signaling Properties of μ-δ Heterodimers. We next examined whether δ receptor ligands are able to enhance the signaling by μ receptors. For this examination, we used the agonist-mediated activation of G proteins, a proximal step in receptor activation, as the assay, and measured the effect of a variety of δ receptor-selective ligands (agonists, antagonists, and inverse agonists) on μ receptor-mediated increase in the binding of [^{35}S]GTPγS (a nonhydrolyzable radiolabeled analogue of GTP; ref. 13). In heterologous cells and neuroblastoma cells coexpressing these receptors, we find a significant enhancement of μ agonist-mediated signaling by all of the δ ligands tested (Fig. 2). A similar increase is also seen in spinal cord membranes (Fig. 3*A*), suggesting that the δ receptor occupancy is sufficient to potentiate μ receptor signaling both in heterologous cells and an endogenous tissue expressing these receptors.

To examine whether this effect can be seen with clinically relevant drugs, we used morphine, fentanyl, and methadone. We find that the δ receptor-selective ligands (agonist and antagonist) are able to significantly enhance signaling by these three drugs (Fig. 3*A* and Table 1). This effect is seen only in membranes from WT mice and not in membranes from mice lacking δ receptors, suggesting that both μ and δ receptors participate in the observed μ–δ synergy (Fig. 3*B*). This finding is consistent with the notion that δ receptor occupancy influences the conformation of the μ receptor, leading to an enhancement in the efficacy of μ receptor signaling.

Because the δ antagonist, TIPPψ, enhances the binding of morphine (Fig. 4*A*) as well as the G protein activation mediated by morphine (Fig. 4*B*), we directly examined whether TIPPψ could affect morphine-mediated decrease in intracellular cAMP levels; activation of opioid receptors leads to a decrease in the level of this classical signal-transducing molecule. We find that the efficacy of morphine for inhibiting cAMP is significantly enhanced by a very low dose (10 nM) of the δ antagonist, TIPPψ (Fig. 4*C*).

Potentiation of Morphine-Induced Analgesia by δ Receptor Antagonist. Next, we explored the physiological consequences of the enhanced binding and signaling by examining the ability of the δ receptor-selective antagonist, TIPPψ, to potentiate morphine analgesia by using the tail-flick assay after intrathecal administration of morphine (14). We focused these studies on the δ antagonist, because the ability of δ agonists to potentiate morphine antinociception at both spinal and supraspinal level is well documented (16–19). Therefore, the use of a δ antagonist rather than an agonist would eliminate any confounding issues with regards to additive effects and allow us to focus on μ-δ modulation.

We find that the analgesia induced by a submaximal dose of morphine can be significantly enhanced by the δ receptor antagonist (Fig. 4*D*). These findings are consistent with those from animals lacking μ receptors, where the δ receptor-mediated analgesia was found to be significantly altered (20, 21). Although these and other previous studies (16–19, 22–25) reported a role for δ receptor ligands in modulating μ receptor-mediated analgesia, the molecular basis for this effect had not been well explored. Here, we show that physical interactions could, at least in part, form the basis for the opiate enhancing effects of δ receptor ligands. Taken together, these studies provide a model for the development of δ receptor ligands that can be used in combination with opiate drugs in the effective treatment of chronic pain.

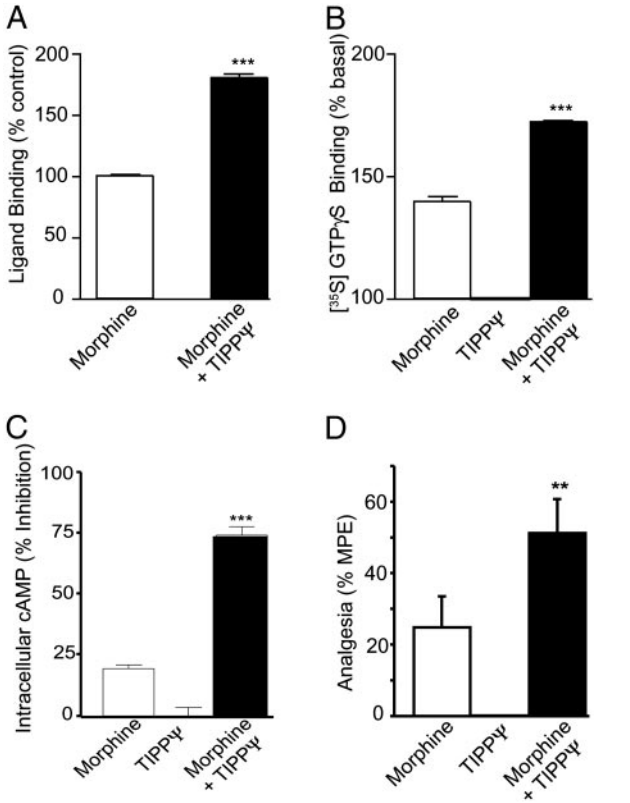

Fig. 4. The δ receptor antagonist enhances μ receptor activity *in vitro* and potentiates morphine analgesia *in vivo*. (*A*) Binding of 6 nM ^{3}H-morphine to SK-N-SH cells endogenously expressing μ and δ receptors in the absence or presence of 10 nM TIPPψ. Specific binding (fmol/mg protein) obtained in the absence of TIPPψ is taken as 100%. Values represent the mean ± SEM of three experiments in triplicate. ***, $P < 0.001$, *t* test. (*B*) Morphine (10^{-7} M)-mediated [^{35}S]GTPγS binding to SK-N-SH cells endogenously expressing μ and δ receptors in the absence or presence of 10 nM TIPPψ. Basal values obtained in the absence of agonist treatment (but presence of antagonist) are taken as 100%. Results are the mean ± SEM of three experiments in quadruplicate. ***, $P < 0.001$, *t* test. (*C*) Morphine (10^{-9} M)-mediated inhibition of the levels of intracellular cAMP in SK-N-SH cells endogenously expressing μ and δ receptors in the absence or presence of 10 nM TIPPψ. Basal values obtained in the absence of agonist treatment (but presence of antagonist) are taken as 100%. Results are the mean ± SEM of three experiments in quadruplicate. ***, $P < 0.001$, *t* test. (*D*) Morphine-mediated intrathecal analgesia in mice. Intrathecal analgesia was measured by the tail-flick assay 30 min after the injection of morphine (0.3 nmol, a dose that gives 20% maximal possible effect) in the absence or presence of 2 nmol of TIPPψ. Results are the mean ± SEM of 20 animals per group. **, $P < 0.01$, *t* test.

Discussion

Morphine, fentanyl, and methadone are clinically relevant drugs. The former two are used in pain management, whereas the latter is used in the treatment of heroin addiction. All three drugs act primarily at the μ opioid receptor. Although previous behavioral studies showed that coadministration of δ receptor ligands could potentiate morphine analgesia, the mechanism was not extensively explored (16–19, 22–25). In this study, we show that δ opioid receptor agonists, antagonists, and inverse agonists modulate μ opioid receptor pharmacology by increasing the number

of binding sites and enhancing the extent of receptor signaling. Most significantly, this study shows that the potency and efficacy of clinically relevant μ drugs can be enhanced by low doses of δ antagonists. This result is potentially of great therapeutic significance because it suggests a strategy for the development of δ receptor-selective ligands that can enhance the effects of clinically used μ drugs with a concomitant decrease in their side effects. One strategy that could be developed is the use of bivalent ligands, where one of the pharmacophores would be a δ receptor antagonist separated from the other pharmacophore, a μ receptor agonist, by a spacer of sufficient length to bridge the two receptors. This approach has been used to synthesize bivalent enkephalin moieties that were shown to display enhanced affinity and selectivity for the δ receptor (26).

The potentiation of μ opioid receptor binding and function by low doses of δ ligands could be accounted for by direct receptor–receptor interactions. This conclusion is supported by our BRET data, which suggest that both receptor types are in close proximity in live cells, immunoprecipitation data, which suggest that the receptors are in interacting complexes, and signaling studies, which show that both receptors are required for the observed synergistic interaction. Binding studies carried out in the 1980s had, in fact, postulated the existence of interacting μ and δ receptor complexes distinct from individual μ and δ receptors (27, 28). It is possible that activation of one of the receptors in the heteromeric complex induces or stabilizes the other receptor's conformation in the active state. This stabilization could, in turn, lead to increased efficacy of G protein activation. It is also possible that in the heteromeric complex each unoccupied receptor acts as a negative modulator of the other's activity, which would be lost after its occupancy by low doses of ligands. Alternatively, heterodimerization could lead to a switch of receptor-associated G proteins to other forms, including pertussis-insensitive G proteins (10, 23, 29–31) or to the release of G proteins sequestered by δ receptors (making them available to the neighboring μ receptors). However, the fact that δ agonist, antagonist, as well as inverse agonist, can increase μ signaling, makes this latter possibility unlikely. Any or a combination of these mechanisms could lead to the observed synergistic interaction. Altered receptor pharmacology due to dimerization/heterodimerization or association with other proteins such as receptor activity modifying proteins has been observed in the case of other GPCRs (9–12, 32–37).

The ability to form heterodimers thus provides an opportunity to explore whether combinations of receptor ligands can be used to modulate therapeutic potential. The studies presented here indicate that knowing what types of heterodimers are formed would allow for the design of therapies that use a combination of receptor ligands as drugs. Our study therefore provides an initial model for the generation of drugs and/or combination therapies not only for the treatment of pain and narcotic addiction but also in a number of disorders where the receptor activity is modulated by heterodimerization/oligomerization.

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